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MATURITY AND SPAWNING PERIOD OF *THRISSOCLES PURAVA* (HAM.) AS DETERMINED BY OVA-DIAMETER MEASUREMENTS

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Introduction

In our studies on the maturity of fishes in Bombay waters, it was noted that the occurrence of *Thrissocles purava* was in two periods, from late January to April and from late August to October, and that the ovaries of specimens obtained in these months were in mature, spent and maturing condition. During these periods the post-larval forms of this fish were also encountered in the plankton.* In the remaining months of the year a few young and immature specimens were caught in inshore waters. This prompted us to examine this species more carefully in order to arrive at some definite conclusion regarding its maturity and breeding season.

Thrissocles purava is a small-sized fish attaining a maximum length of 270 mm. and weighing about 140 gm. The average commercial size is about 230 mm. weighing about 85 gm. It is known locally as "Kateri", on account of the presence of a number of small bony spines in its skeleton. This fish is generally consumed by the lower middle class and poor people.

* 3rd "Progress Report on 'Investigation of fish eggs & fish larvæ from Bombay waters' 1946-47," I.C.A.R., Fisheries Scheme, New Delhi.

MATERIAL AND METHODS

A representative collection of varied sizes was obtained twice or thrice a week from fish markets where fishes were brought from the neighbouring fishing centres. After bringing the fish to the laboratory, the specimens were cleaned and their total body-length and weight were taken. The fish were then cut open ventrally and the general appearance and the extent of the ovary in the abdomen were noted. The ovaries were taken out carefully and weighed. A small portion of the ovary was teased on a slide and examined under the microscope and the different stages of ova were noted. The ovaries were subsequently fixed in 10% formalin and allowed to harden for several days before the ova-diameters were measured. A record was maintained of the locality of the fish caught, their length and weight, the size and weight of the ovary and the state of maturity, in a tabular form throughout the period of this investigation (1947–48).

Measurement of ova-diameters.—At least three or four ovary samples from maturing and mature specimens, were taken for measurement of ova-diameters. The anterior, the middle, and the posterior portions of the preserved ovaries were examined. The distribution of ova of various sizes was found to be uniform throughout the entire mass of the ovary. As no appreciable shrinkage was noticed in the preserved material, the latter could be conveniently used for the purpose. The ova-diameters were measured by means of a micrometer eye-piece at a magnification which gave a value of $16{\cdot}9\,\mu$ to each micrometer unit. For the estimation of the size-frequency percentages of ova, about 300 ova from each ovary sample of maturing and mature fish were measured. The diameter counts were arranged into different groups and their corresponding percentages were calculated.

Enumeration of mature ova.—The enumeration of mature ova produced for a single spawning was done by what is known as *specific-gravity-bottle method.* The procedure adopted was as follows:—

(1) Weight of the specific gravity bottle.. .. .. W

(2) Weight of the specific gravity bottle filled with water .. W_1

(3) Weight of the specific gravity bottle filled with mature ova .. .. W_2

(4) Weight of the specific gravity bottle filled with mature ova and water .. .. W_3

∴ (5) Weight of water (in sp. gr. bottle) .. .. .. (W_1-W)

(6) Weight of water not occupying the volume of mature ova .. .. (W_3-W_2)

∴ (7) Weight of water occupying the volume of mature ova .. .. .. $(W_1-W)-(W_3-W_2)$

(8) = Volume of water displaced by the ova.

= Volume of the mature ova.

Since, volume of one mature ovum = $4/3\pi r^3$.

Total number of mature ova =

$$= \frac{\text{total volume of mature ova}}{\text{volume of one mature ovum}}$$

$$= \frac{(W_1 - W) - (W_3 - W_2)}{4/3\pi r^3}$$

From the formula, the total number of mature ova present in the entire ovaries of the mature fish was calculated.

Growth of Ovary from Immature to Mature Stage

Before describing the successive changes in the ovary, it is advisable to state precisely the meaning of the terms immature and mature. It must be remembered that the term immature may mean a young fish which had never spawned before, or it may also refer to older fish which, on account of the immature condition of the ovary, can still be called immature. The term mature may be applied to a fish becoming mature for the first time, or to a fish attaining maturity subsequent to the first spawning.

In the present description, the term immature is applied to a fish containing immature ovaries, with ova smaller than 0·45 mm. A maturing fish has its ova ranging from 0·45 mm. to 0·76 mm.; while a mature fish has them from 0·92 mm. to 1·26 mm. in diameter.

Immature fish and its ova.—As stated previously, the immature specimens were encountered in very small numbers in the inshore catches. They measured about 140–50 mm. on an average. The ovaries of an immature fish are in the form of two longitudinal cream-coloured strips attached to the ventral side of the air-bladder, and extend over $\frac{1}{3}$ to $\frac{1}{2}$ the length of abdomen. The right ovary is relatively smaller than the left one. They measure from 20 to 30 mm. in length and weigh from 0·5 gm. to 1·5 gm.

The ova contained in these ovaries are primarily in the immature condition. They measure from 0·08 mm. to 0·44 mm. in diameter. Of these the smaller ones with a diameter varying from 0·08 to 0·33 mm. appear as quite transparent, spherical cells, containing a distinct nucleus. In-between hese ova which form the bulk of the ovary, there may also be found, a few

larger ova which exhibit a tendency towards yolk formation. In these larger immature ova, whose diameters vary from 0·33 to 0·44 mm. a thin layer of very minute yolk granules is being gradually developed along the periphery of the cell wall, which marks the initial step in the deposition of yolk in an immature ovum. By reason of the presence of these developing yolk granules these ova present a more or less semi-transparent appearance. The presence of such semi-transparent ova in the immature ovary is indicative of its gradual development towards the maturing stage.

Maturing fish, its ovaries and ova.—Maturing females were noted in fairly large numbers during the periods of the occurrence of the species. In specimens measuring from 174 mm. to 244 mm. and weighing from 41 gm. to 89 gm. the ovary length varied from 40 mm. to 64 mm. and its weight from 2 to 5·8 gm.

The maturing ovaries contain two types of ova, namely the immature ones described above and the maturing. The diameters of the latter vary from 0·45 to 0·76 mm. Of these, the ova smaller than 0·50 mm. in diameter are partially opaque, being more opaque at the periphery than in the central zone. Ova with a diameter varying from 0·50 to 0·76 mm. represent the typical maturing ova, by virtue of their being densely packed with yolk granules and are therefore highly opaque in appearance.

Mature fish, its ovaries and ova.—A mature fish is easily recognisable by its distended abdomen, due to the extensive growth of the ovaries. Mature specimens measured from 188 mm. to 240 mm. in length and weighed between 51 gm. and 94 gm. Their ovaries varied from 55 mm. to 75 mm. in length, and from 12 gm. to 15·5 gm. in weight. A single mature fish, 270 mm. long and weighing 140 gm., was encountered only once. Its ovaries, 75 mm. long, weighed as much as 20 gm.

An examination of the mature ovary under the microscope shows the presence of two types of ova: mature and immature, the latter forming but a small amount of the bulk of the ovary. A mature ovum is spherical in shape with a diameter varying from 0·92 mm. to 1·26 mm. It is transparent in appearance and the yolk is in the vacuolated condition. The oil globules are absent in the ovum of this species.

Spent fish.—As stated previously, the mature and the spent fish occur simultaneously in large numbers from February to April and from August to October. These two periods obviously represent the spawning seasons of this species. The spent ovaries, weighing 1·2 to 4·2 gm. are flaccid, wrinkled and slightly reddish in appearance. Empty follicles from which

mature ova have been extruded, are found in plenty in these spent ovaries. After going through the process of degeneration of the empty follicles, the spent ovaries become reorganised. With the addition of a fresh crop of immature ova, the old ones already present in them begin to grow into the maturing stage in due course. Such specimens with maturing ovaries are frequently met with in each season. They are in the process of preparing a new crop of mature ova for the next spawning season which would follow after about six months.

The Total Number of Mature Ova Produced for a Single Spawning

The enumeration of ova was done with specific-gravity-bottle method, and the total number of ova produced for one spawning was found to vary with the size of the fish, there being usually more ova in a larger specimen. The size of the fish and the total number of mature ova produced are given below:—

Size of fish	Number of mature ova
170 mm. ..	.. 5,842
187 mm. ..	.. 11,896
192 mm. ..	.. 15,175
205 mm. ..	.. 17,457
207 mm. ..	.. 19,490
212 mm. ..	.. 21,721
222 mm. ..	.. 22,485
237 mm. ..	.. 23,878

Size-Frequency Percentages of Ova from Mature Fish

In the description of the mature ovary it has been pointed out that the mature ovary contains two types of ova: immature and mature. In order to determine the proportion of each type, the size-frequency percentages of immature and mature ova were recorded in a tabular form and also shown graphically. The isolation of these groups by the total absence of the maturing ova is clearly seen in Table I and Figs. 1, 2. An analysis of the size-frequency percentage data in Table I brings to light the following important points.

(i) The percentage of immature ova varies between 30% to 50%.

(ii) The percentage of mature ova is from 50% to 70%.

Figs. 1 and 2 clearly indicate the percentage distribution of ova in mature ovaries and also show two distinct modal points in each of them. The first modal point varies from 0·21 mm. to 0·38 mm In the majority

TABLE I

Size-Frequency Percentages of Ova in the Mature Ovaries (1947–48)

Diameter ranges of ova in millimeters	Ova percentages					
	August	September	October	February	March	April
0·17–0·26 ..	10·4	4·0	12·7	11·2	8·8	10·2
0·26–0·34 ..	9·3	27·0	13·5	11·6	9·0	29·5
0·34–0·43 ..	28·7	6·0	13·0	5·6	23·1	3·0
0·43–0·51 ..	3·8	5·6	3·3	0·0	0·8	0·0
0·51–0·59 ..	0	0	0	0	0	0
0·59–0·68 ..	0	0	0	0	0	0
0·68–0·76 ..	0	0	0	0	0	0
0·76–0·85 ..	0	0	0	0	0	0
0·85–0·93 ..	0·0	4·8	8·3	1·2	1·3	1·0
0·93–1·02 ..	0·3	33·3	27·8	36·2	37·2	21·4
1·02–1·10 ..	1·7	18·5	16·2	34·1	19·7	34·6
1·10–1·19 ..	35·9	0·8	5·2	0·0	0·0	0·3
1·19–1·27 ..	9·9	0·0	0·0	0·0	0·0	0·0

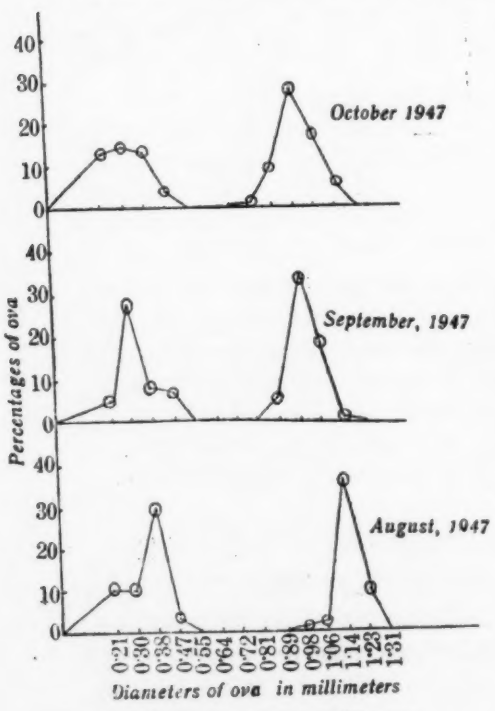

FIG. 1. Diameters of ova in millimeters.

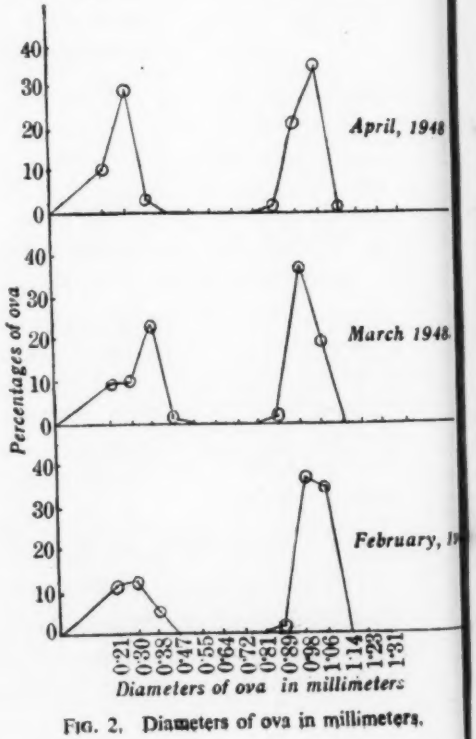

FIG. 2. Diameters of ova in millimeters.

of polygons, it remains at 0·30 mm. It represents the group of *immature* ova, which ranges from below 0·21 mm. to 0·49 mm. The second modal point varies from 0·98 mm. to 1·14 mm. In the majority of polygons, it represents the group of *mature* ova, which generally begins at 0·92 mm. and extends as far as 1·26 mm. These polygons bring to light two notable features of this fish:—

(i) The complete separation of the mature ova group from the immature one.

(ii) The complete absence of the maturing ova in the mature ovary.

Spawning of *Thrissocles purava*

It has been stated at the outset that this species is known to occur in Bombay waters mainly during two periods of the year: once from late January to April and again from late August to October. At other times the adult fish moves elsewhere from the Bombay shores.

During the periods of its occurrence in Bombay waters, the adults are found in plenty in maturing, mature and spent condition of their ovaries, while only a small percentage, with immature ovaries is known to visit the shores. On analysis of the available material, the percentage of the adult females in each stage of maturity, during the period under investigation, is given in Table II.

Table II

Percentage of Females in Each Stage of Maturity

Stage of Maturity	(1947–48)						
	Aug.	Sept.	Oct.	Jan.	Feb.	Mar.	Apr.
Immature ..	15	7·7	6·1	10	11·1	6·4	0
Maturing ..	50	15·4	15·3	90	33·3	32·3	0
Mature ..	35	61·5	52	0	38·9	41·9	75
Spent ..	0	15·4	26·5	0	16·4	19·4	25

The figures given above point out the following noteworthy features in respect of the spawning activity of the species:—

(i) Mature and spent specimens occur plentifully during late August to October and from February to April.

(ii) The peak spawning occurs during September–October and February–April months.

(iii) The percentage of the spent females shows an increase at the close of the spawning season.

(iv) The percentage of the maturing females is fairly large during the spawning period.

(v) The percentage of the immature females is very low during the two periods of occurrence of this species.

It will be seen from the above that the simultaneous occurrence of the mature and spent specimens during August–October and February–April periods, indicates that the fish spawns at that time and that it visits the Bombay shores for that purpose.

DURATION AND FREQUENCY OF SPAWNING

From the knowledge of the structure of the mature ovary and the nature of the catch during the spawning periods, there is some evidence to indicate that the spawning of *Thrissocles purava* is of a short duration and that it spawns only once during the season. The fact that the mature ovaries contain only two types of ova namely mature and immature (Table I) and that these two groups are completely separated from each other (Figs. 1, 2), suggests according to Rutenberg and Hickling (1936), a discontinuous growth of ova and therefore a short spawning period. Further the plentiful occurrence of mature fish coupled with the increasing proportion of spent females during the spawning seasons, indicates, according to Walford (1932), that the individual fish may have only one spawning during each season.

FATE OF THE MATURING OVA

The fish after spawning remains in the inshore waters and leaves the shore at the close of the spawning season, with its ovaries in the maturing stage. The maturing fish which continued to appear throughout the spawning period, cannot be expected to mature during that season as can be seen from the size-frequency percentage data in Table III.

The data in Table III are represented by polygons in Fig. 3. A study of the polygons indicates two distinct modal points in each. The first is located at 0·21 mm. and represents the group of *immature* ova, which extends as far as 0·45 mm. The second modal point is located at 0·55 mm. and represents the group of *maturing* ova, whose upper limit extends as far as 0·68 mm.

It must be made clear that the readings in Table III represent only a sample of maturing ovaries obtained during the spawning seasons. They seem to corroborate the view that the maturing specimens which are present

TABLE III

Size-Frequency Percentages of Ova in the Maturing Ovaries

Diameter ranges of ova in millimeters	Percentages of ova in different seasons	
	21-10-1947	20-4-1948
0·17–0·26	15·3	25·2
0·26–0·34	12·8	20·0
0·34–0·43	3·8	5·6
0·43–0·51	2·9	9·6
0·51–0·59	40·9	36·8
0·59–0·68	24·3	2·8

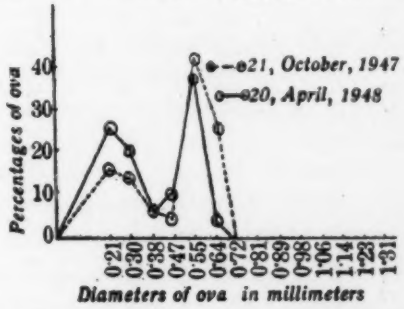

FIG. 3. Diameters of ova in millimeters.

in fairly large numbers in the seasonal catches, are not likely to mature in the same season, as they contain only the small maturing ova varying from 0·45 mm.–0·64 mm. It appears quite possible, however, that this (maturing) stock, which disappears from the local habitat at the close of the season, becomes mature and then returns to Bombay waters during the next season for spawning. Since the time interval between two successive spawnings is about six months, the latter seems to be the minimum period required for the growth and rematuration of ova after each spawning.

SIZE AT FIRST MATURITY

The data employed for the determination of minimum size at maturity are based on 360 fish obtained from Sassoon Docks and Dadar fish market. Since the study relates to female fish, the conclusions drawn apply to that sex only. The simultaneous presence of mature and spent specimens in

the catch obviously indicate that they were the individuals involved in spawning activity during the season; and hence only such specimens were taken into consideration for determination of size at first maturity.

The number and percentage of mature and spent fish grouped by 5 millimeter length classes are given in Table IV. The data are shown by histograms in Fig. 4. They indicate that,—no mature fish were found with a

TABLE IV

Number and Percentage of Mature and Spent Females (Thrissocles purava) *in each 5 Millimeter Length*

Total length in millimeters	Total fish examined	Number mature and spent	Per cent. mature and spent
150–154 ..	6	..	..
155–159 ..	2	..	..
160–164 ..	9	2	22·2
165–169 ..	3	1	33·3
170–174 ..	5	5	100·0
175–179 ..	15	8	53·3
180–184 ..	36	30	88·4
185–189 ..	59	45	76·2
190–194 ..	18	18	100·0
195–199 ..	13	8	61·5
200–204 ..	43	27	62·8
205–209 ..	27	27	100·0
210–214 ..	45	42	93·3
215–219 ..	15	15	100·0
220–224 ..	16	8	50·0
225–229 ..	9	00	00
230–234 ..	4	00	00
235–239 ..	24	9	37·5
240–244 ..	8	4	50·0
245–249 ..	0	0	0
250–254 ..	2	2	00·0
255–259 ..	..	..	..
260–264 ..	..	..	..
265–269 ..	..	..	..
270–274 ..	1	1	100·0

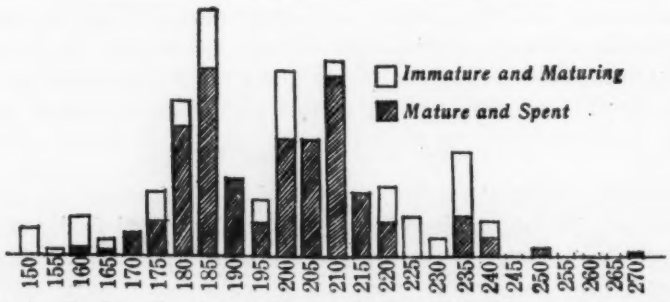

FIG. 4. Number of mature and spent females (*Thrissocles purava*) in each 5 millimeters of length.

length less than 160 mm.; = 22·2 per cent. of the females was mature between 160–64 mm.; —33·3 per cent. between 165–69 mm. and a major per cent. was mature in practically all the length classes above 170 mm. It may therefore be stated with some degree of precision that the female *Thrissocles purava* mature at an average length of 170 mm.

Summary

The peak period of occurrence of *Thrissocles purava* (Ham.) in Bombay waters is in two seasons, namely February-April and August–October. The mature, spent and maturing fish are caught in inshore waters in these periods. The post-larval forms are also encountered in the plankton samples taken in these months. During the remaining months a few young, immature specimens are available in small numbers.

The range of diameters of immature, maturing and mature ova is from 0·08 mm. to 0·44 mm.; 0·45 mm. to 0·76 mm. and 0·92 mm. to 1·26 mm. respectively.

The mature ovaries of this species are found to contain only two groups of ova, namely mature and immature.

The spawning season, as determined by the ova diameter measurements and the structure of the mature ovary, is found to be the same as the peak period of occurrence of this species in inshore waters. Relatively high percentages of mature and spent females during this period further supports the view that the species visits the Bombay shores mainly for spawning.

The spawning period for individual fish is of a short duration and there is only one spawning a season. The minimum period required for the rematuration of the spent ovaries is about six months.

The size at first maturity is found to be about 170 mm.

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STUDIES ON THE MUTAGENIC ACTION OF CHEMICAL AND PHYSICAL AGENCIES ON YEASTS

V. Effect of Ultraviolet Irradiation on a Diploid Brewery Yeast

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Introduction

Reversal of gene mutations (Subramaniam and Krishna Murthy, 1948) as well as an induction of tetraploidy (Ranganathan and Subramaniam, 1950) was observed in this laboratory when our two chromosome brewery yeast was exposed to ultra-violet irradiation. Recent investigations revealed the mutagenic action of polyploidogens on yeast. It has been shown that camphor (Subramaniam and Sreepathi Rao, 1950; Ranganathan and Subramaniam, 1950), colchicine (Ranganathan and Subramaniam, 1950), acenaphthene (Duraiswami and Subramaniam, unpublished) and chrysene (Mitra and Subramaniam, 1949, 1950) like thermal shocks (Ranganathan, 1950; Subramaniam and Prahlada Rao, 1951) produce not only a doubling of the chromosome number but accelerate also the mutations at the various loci. The action of polyploidogens, therefore, does not consist in a mere duplication of the chromosome number, but involves, primarily, a gene mutation enabling such a doubled complement to function harmoniously. It has been conceived that there should be a locus for polyploidy and that the presence of different alleles at this locus governs the viability of specific chromosome numbers. Indirect evidence in support of the above

contention is afforded by the results from irradiation experiments. Chromosomal variants as well as polyploid and haploid types have arisen as a result of irradiation. Goodspeed (1936) in fact suggests that the alterations in the chromosome numbers may be the result of induced gene mutations. If that is so, tetraploidy in yeast should be sporadic and exposure to ultra-violet light need not always result in an induction of polyploidy. The experiments reported here were planned to check the above possibility.

Another interesting fact appears to be the similarity in the explanations offered for the mutagenic action of chemical and physical agencies. Chrysene which induces tetraploidy accelerates the rate of gene mutations. Ultra-violet irradiation which is said to accelerate gene mutations has been shown to induce tetraploidy in our control strain.

MATERIAL AND METHODS

To facilitate correct interpretation of the data, it was thought desirable to start with a diploid culture pure for a given type of sculpturing of the giant colony. Photo 1 shows the appearance of a colony of the control diploid brewery yeast inoculated on 30–8–1949 and photographed on 13–9–1949 at the end of 14 days' growth. It is pure for the *Rough II* type of sculpturing. A loop of material was taken from the periphery of the above colony and transferred to 5 ml. of barley malt wort in a bacteriological test tube. This constituted the starting material for the present investigation and was labelled BY 1S. After allowing the above liquid culture to grow for 24 hours, loops were transferred to two sterile vitreosil assay tubes each containing 2·5 ml. of wort. Enough number of loops were inoculated into each of the tubes to give a good turbidity, thus providing for a high survival percentage after the irradiation.

The two assay tubes were next placed at a distance of 90 cm. from an ordinary laboratory mercury arc lamp and irradiated for 4 hours. Care was taken to see that uniform suspensions of the culture were exposed by periodic shaking. The small diameter of the tubes ensured against screening effects due to the thickness of the suspension. At the end of the irradiation, the cultures were labelled UV 1 and UV 2, shaken well and left inside a sterile chamber to proliferate. After 24 hours they were again shaken well and inoculations in duplicates were carried out for giant colonies. At the same time, colonies of the control BY 1S were also run from a 24-hour old liquid culture. These are illustrated in Photos 2, 3, 6, 7, 8 and 9.

The cultures UV 1 and UV 2 were given a change of medium immediately after the inoculations for the giant colonies. Six hours later, two loops from each of the well shaken cultures were inoculated into fresh tubes of wort. The material obtained in each case at the end of growth for 16 hours was plated out by a routine procedure followed in our laboratory. To assess whether any variants had appeared in the control, a 16-hour old culture of BY 1S was also plated out at the same time. After the lapse of a week, two colonies of the control and four each from the plates of UV 1 and UV 2 were picked out on the basis of morphological characteristics for detailed investigation. Thus there were two Controls, 1 and 2, and eight cultures derived from the irradiated material, CYG 1–4 from UV 1 and CYG 5–8 from UV 2. The following table gives in detail all the relevant data:—

Strain	Source	Morphological Characteristics
Control 1	Dilution plate	Circular colony, wavy margin, rough.
Control 2	of BY 1 S	Circular colony, entire margin, smooth.
CYG 1		Big, circular, rough colony, wavy margin.
CYG 2		Small, heart-shaped, rough colony, wavy margin with a notch at the centre.
	Dilution plate	
CYG 3	of UV 1	Big, microscopically rough but macroscopically smooth colony.
CYG 4		Small, smooth colony.
CYG 5		Smoothish colony, big, circular, entire margin.
CYG 6	Dilution plate	Small, smoothish, yellowish colony.
CYG 7	of UV 2	Very small, slightly notched colony.
CYG 8		Big, smoothish colony, entire margin.

After growth for 24 hours in wort, the above strains were transferred to agar slants. A week later, these were tested for their giant colony characteristics by inoculations from 16-hour old wort cultures. The colonies of CYG 1 and 5 got contaminated during the early phases of growth and as such had to be rejected. The others are illustrated in Photos 4, 5, 10, 11, 12, 14, 15 and 16.

To test whether delayed effects due to the irradiation could be observed, the giant colony characteristics of the above strains were checked along with those of the controls after more than a month. These inoculations were performed successively for the various strains and Photos 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26 illustrate the colonies obtained.

OBSERVATIONS

At the start of the experiment, the control had the *Rough II* type of sculpturing (Photo 1; *cf.* Photo 10, Subramaniam, Ranganathan and Krishna Murthy, 1948; Photos 9 and 10, Duraiswami and Subramaniam, 1950) and had no sectors. The mark left by the loop when material was removed from this colony could be seen as a dent in the margin. When grown for 24 hours in wort, the culture had become a mixture. The composite type of sculpturing observed in Photos 2 and 3 is the result of the mutants composing them having an identical growth rate. When they have different growth rates, the colonies are sectored. This curious feature has been repeatedly observed in this laboratory. From dilution plates the types could be isolated separately. Photos 4 and 5 show the two mutants responsible for the mixed nature of the sculpturing in Photos 2 and 3. The colony in Photo 4 is of the characteristic *Smooth III* type (*cf.* Photo 14, Subramaniam, Ranganathan and Krishna Murthy, 1948), while that in Photo 5 could be assigned to the *Rough I* class. The slightly different appearances of Photos 2 and 3 are probably the result of the two mutants not occurring in the same proportion. It is these types which have been exposed to the action of ultra-violet light. Photos 6 and 7 illustrate the giant colonies grown from the first tube labelled UV 1. Both belong to the *Rough* category with a rim (*Rough III*). In Photo 6 there are a few *Smooth* sectors one of which alone starts from the centre. Photo 7 on the other hand is pure for the type. The *Rough III* (Krishna Murthy and Subramaniam, 1950) has been assumed to be of the genic constitution *Rough/Rim*. The cells before irradiation had the genic constitution *Lace/Lace* (*Smooth III*, Photo 4) and *Rough/Smooth* (*Rough I*, Photo 5). After irradiation the genic constitution has changed to *Rough/Rim*. Ultra-violet irradiation should therefore have produced the following alterations:—

Smooth III	Lace	→ mutation →	Rough	*Rough III*
	Lace	→ mutation →	Rim	
Rough I	Smooth	→ mutation →	Rim	*Rough III*
	Rough	——————→	Rough	

The changes in the second irradiated culture, UV 2 are not strictly comparable to the previous one. Photo 8 is of the *Rough II* category, but it also shows a broad *Smooth* sector extending from the centre to the periphery. From its shape it could be presumed that its rate of growth is inferior to the rest of the colony. This *Smooth* sector could have originated either from the *Smooth III* or from the *Rough I* cells present before the irradiation.

$$\textit{Smooth III} \begin{cases} \text{Lace} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Lace} \rightarrow \text{mutation} \rightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

$$\textit{Rough I} \begin{cases} \text{Rough} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Smooth} \longrightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

A single gene mutation would convert the *Rough I* into the *Smooth I* type. The *Smooth I* sector is likely to have originated, therefore, from the *Rough I* cells. Photo 9 on the other hand, resembles the control at the start of the experiment (Photo 3). It also shows a *Smooth I* sector.

Immediately after the giant colony inoculations of the irradiated material, the cultures were plated as described before. The strains isolated from the plates were now tested for their giant colony characteristics. The colony of CYG 1 got contaminated and had to be rejected. CYG 2 developed into a colony as illustrated in Photo 10. Its sculpturing is reminiscent of the *Smooth III* condition, but it does not appear to be pure for the type. The minute granules scattered especially in the outer half of the colony suggest that the Rough cells are persisting. Before plating, the material was of the *Rough III* type (Photos 6 and 7). During proliferation after irradiation, therefore, mutations should have been going on, reversing the changes induced by exposure to ultra-violet. This phenomenon is much more pronounced in the strain CYG 3 (Photo 11) which has reverted to the condition of the control before treatment with the radiation (*cf.* Photo 3). It is quite likely that the action of ultra-violet consists merely in a temporary acceleration. The increased rate of mutability of the locus seems to persist for some time. While in CYG 3 the reversion was to the original condition, CYG 4 confirms the belief that mutation need not always be in the reverse direction. The colonies illustrated in Photos 12 and 13 are perfectly smooth and entirely different from the control and the other strains isolated from the culture UV 1. Immediately after irradiation, the colony was composed of *Rough III* cells. The following changes should therefore have occurred when cultured after exposure:—

$$\textit{Rough III} \begin{cases} \text{Rough} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Rim} \rightarrow \text{mutation} \rightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

The four strains isolated from the second irradiated tube, UV 2, show a different type of change. The giant colony of CYG 5 got contaminated and had to be discarded. CYG 6 gave a characteristic *Rough I* colony which had a broader sculptured outer region. It is pure and shows no sectors. Immediately after irradiation UV 2 was a mixture of *Rough III*, *Rough I*, *Smooth III* and *Smooth I* categories of cells. It is difficult therefore to evaluate the exact changes. CYG 7 (Photo 15) gave a *Rough I* colony in which the granulations obscured the radial and concentric folds.

CYG 8 (Photo 16) is comparable to CYG 2 (Photo 10) in that it is predominantly composed of *Smooth III* type with a sprinkling of *Rough* cells.

A fresh experiment was necessitated because some of the giant colonies of the irradiated strains got contaminated. The *Smooth III* colony illustrated in Photo 4 gave a mixed type (Photo 17) just like the control at the commencement of the experiment (Photo 3). The *Rough I* (Photo 5) colony was succeeded on the other hand by a *Rough II* type (Photo 18) at the same time. So, even in the control cultures the changes are not in an identical direction. The colony of CYG 1 (Photo 19) could be classified as *Rough I* but it does not seem to be pure for the type. There are *Smooth* sectors, one of them originating right from the centre. CYG 2 immediately on isolation from plates was predominantly *Smooth III* in character (Photo 10). After about 50 days, it had become a mixture (Photo 20; *cf.* Photo 3). The composite nature of CYG 3 (Photo 11) changes into a *Rough I* type of colony in Photo 21. Mutation seems to have taken place in CYG 4 also. The pure *Smooth I* condition (Photo 12 and 13) has given place to a mixed type in Photo 22. In CYG 5 too, a similar condition is met with (Photo 23). CYG 6 which was originally *Rough I* (Photo 14) has become transformed into the *Rough II* type (Photo 24) after the lapse of about 5 weeks. It shows a few *Smooth* sectors. It is curious that while pure types become mixtures, mixed types become pure. This periodic fluctuation in the population is further evidenced by the changes in CYG 7. The *Rough I* condition illustrated in Photo 15 has given place to the mixed type in Photo 25. The strain CYG 8 which was predominantly *Smooth III* in character (Photo 16) has been replaced by *Rough II* cells (Photo 26).

DISCUSSION

(*a*) *The nature of mutations induced by ultra-violet*

Ultra-violet irradiation is in general assumed to produce gene mutations, while chemical polyploidogens have been used to induce a doubling of the chromosome complement. The results obtained in this laboratory were rather surprising. Exposure to ultra-violet irradiation produced not only gene mutants (Subramaniam and Krishna Murthy, 1948) but also tetraploids (Ranganathan and Subramaniam, 1950). Polyploidogens like acenaphthene (Subramaniam, 1947) and camphor (Ranganathan and Subramaniam, 1950) induced gene mutations apart from a doubling of the chromosome complement. This led to the suspicion that any induction of polyploidy should itself be preceded by a gene mutation. If that is so, the occurrence of polyploidy after ultra-violet irradiation should be as unpredictable as induc-

tion of any specific gene mutation. In accordance with the theoretical expectations, while gene mutations were induced polyploidy did not occur in this series. Gene mutations are reversible. Demonstration of such a reversibility (Subramaniam, Ranganathan and Krishna Murthy, 1948) led to a scepticism regarding the claim of successive mutations described by Skovsted in *Nadsonia* (Skovsted, 1943). At the same time reverse mutations also offered proof for the existence of multiple alleles at the locus governing the nature of sculpturing of the giant colonies. Further support for the above contention is afforded by the results recorded in the present paper.

(*b*) *Effect of ultra-violet rays on higher plants and micro-organisms*

There is a close similarity in the action of ultra-violet radiation on higher plants and micro-organisms. Depending on the dosage, an organism may be killed or a lethal gene mutation producing the same end result may occur. The breakage of the chromosomes and the consequent non-viability of such cells is another factor leading to the death of the micro-organism. Another important factor is, of course, the actual physiological state of the cells at the time of exposure. Fermenting yeast cells which are endopolyploid are on the road to death and final disintegration. Exposure of a purely fermenting culture may not therefore give a correct idea of the lethal dosage.

The prolongation of the lag phase and the persistence of the cells in the prophase condition for a long time has been surmised to be caused primarily by an upset of the nucleic acid cycle (Catcheside, 1948). The breakage of the chromosome at the higher dosage levels is a necessary corollary. A clear distinction has to be made between chromosomal breakages resulting in viable translocations and those in which no such reconstruction is possible. Some of the chromosomal translocation mutants obtained from our control strain by exposure to acenaphthene and thermal shocks have been shown to be viable (Ranganathan, 1950; Ranganathan and Subramaniam, 1950). In fact the evidences indicate that for breakages leading to viable translocation mutants to occur, not only a particular allelic sequence but also one or more specific gene mutations should have preceded a breakage. Exposure of the same strain to the identical agencies after a lapse of 12 to 24 months did not produce the same translocation mutants. The allelic sequences should be in a state of flux owing to the mutations at different loci. A specific mutation leading to a viable chromosomal translocation is surmised to occur only under very definite conditions. Whether exposure to ultra-violet would produce such mutants was also kept in view during these experiments, for the top yeasts so obtained are

not only purely aerobic but excrete considerable amounts of riboflavin (Mitra, 1949). No such top yeast was obtained in the present investigation.

(*c*) *Mode of action of physical and chemical mutagens*

Investigators on chemical mutagens have been struck by the similarity in the action observed to those recorded after exposure to ionizing radiations. Though Dustin (1947) has attempted to classify the chemical mitotic poisons into the colchicine and trypaflavine types, this distinction is found to break down when it is realised that urethane, classified under the latter category, has been shown to be mutagenic (Oehlkers, 1943, 1946, 1949; Vogt, 1948). It appears probable that mitotic poisons may be mutagenic at lower concentrations. Mutagens at higher levels will necessarily have lethal effects. The surprising fact that emerges from the extended observations on the effect of physical and chemical agencies on yeasts is that

Agent	Gene mutations	Chromosomal translocations	Polyploidy	Authors
Acenaphthene	+	+	+	Ranganathan and Subramaniam, 1950
Do. ..			+	Subramaniam and Krishna Murthy, 1949
Do. ..	+			Duraiswami and Subramaniam, unpublished
Thermal shocks	+	+	+	Subramaniam and Ranganathan, 1947; Ranganathan, 1950
Do. ..	+			Subramaniam and Prahlada Rao, 1951
Chrysene ..			+	Mitra and Subramaniam, 1949
Do. ..	+		+	Idem, 1950
Camphor ..	+			Subramaniam and Sreepathi Rao, 1950
Do. ..			+	Ranganathan and Subramaniam, 1950
Ultraviolet ..	+			Subramaniam and Krishna Murthy, 1948
Do. ..			+	Ranganathan and Subramaniam, 1950
Colchicine ..			+	Ranganathan and Subramaniam, 1950

mutagens are capable of inducing polyploidy under specific conditions, while polyploidogens do produce gene mutations. This would become apparent from the above table:—

The only conclusion that could be drawn from the observations tabulated above is that both physical and chemical agencies should have a common mode of action. Such a contention receives further support from published literature. The consensus of opinion is that the observed effects of radiations are due to a transfer of energy to the cell (Catcheside, 1948; Lea, 1946). Auerbach (1949 *a*) considers that there is considerable experimental evidence for Jordon's "Treffergifte" theory according to which chemical mutagens simulate the radiations in that they exert their effects by "localized contacts with certain governing centres of cell activity" (Auerbach, 1949 *a*). What exactly happens when such contacts occur? The retardation of cellular division observed after *x*-irradiation has been attributed to the upset in the nucleic acid cycle of the cell. A dislocation in the normal metabolism of the cell has thus been brought about. On the basis of such an upset, it is possible to understand why ordinary physiological conditions like age or sex of the material should affect the spontaneous mutation rates in animals. Auerbach herself visualizes the possibility that "Treffergifte" agents could act through a change in the metabolic pattern of the test organism. In this connection the results obtained by Wyss, Stone and Haas (1947) are worthy of consideration. They found that when *Staphylococcus aureus* was allowed to grow in a medium containing known substrates which had been subjected to irradiation with ultra-violet, mutation rate to penicillin or streptomycin resistance could be increased. It is presumed that the mutations are caused by the activated chemicals supplied as substrates to the organism. It appears feasible that utilisation of such an activated chemical by the cells will involve a change in the normal metabolic pattern and the observed mutations could therefore be attributed to the upset in the metabolism of the organism. Results obtained by Auerbach (1949 *b*) on the mutagenic ability of formalin also point to such a conclusion.

Such an interpretation would render possible an alignment of the observed effects of cold shock reported by many investigators. Any thermal shock should result in metabolic derangements. Looked at from this angle, the differences between the various chemical and physical agencies would be only one of degree. An upset in the metabolism could then be effected by action either on the nucleus or the cytoplasm.

The underlying causes for spontaneous gene mutations have been a matter of speculation ever since gene mutations themselves were dis-

covered. Attempts have been made to attribute any agency which has been known to accelerate the mutation rate as the primary causative agent. Reasons have been adduced to show that the acceleration in the mutation rate by physical and chemical agencies should primarily be traced to disturbances in metabolism. If the above contention is accepted, any drastic alteration in the metabolic activity of the organism should produce gene mutations.

SUMMARY

1. Investigations in this laboratory indicated that polyploidogens are also mutagenic and that mutagens may induce polyploidy. Doubling of the chromosomes leading to viable tetraploid races is sporadic and such an induction was observed after ultra-violet irradiation.

2. Experiments were therefore carried out to confirm the suspicion that a doubling of the chromosome complement after ultra-violet irradiation may be as sporadic and unpredictable as induction of a specific gene mutation.

3. Photographs of giant colonies of the treated and the control material are presented.

4. The evidence recorded in this paper confirmed the suspicion that induction of tetraploidy being conditioned by a specific gene mutation and induced gene mutations being sporadic, treatment with ultra-violet rays does not always lead to the production of tetraploidy.

5. The existence of multiple alleles at the locus governing the nature of sculpturing of the giant colony is confirmed.

6. Ultra-violet irradiation has immediate as well as delayed effects and the nature of the mutation induced is unpredictable.

7. A theory of the mode of action of the various biologically effective physical and chemical agents on living organisms is considered.

ACKNOWLEDGMENT

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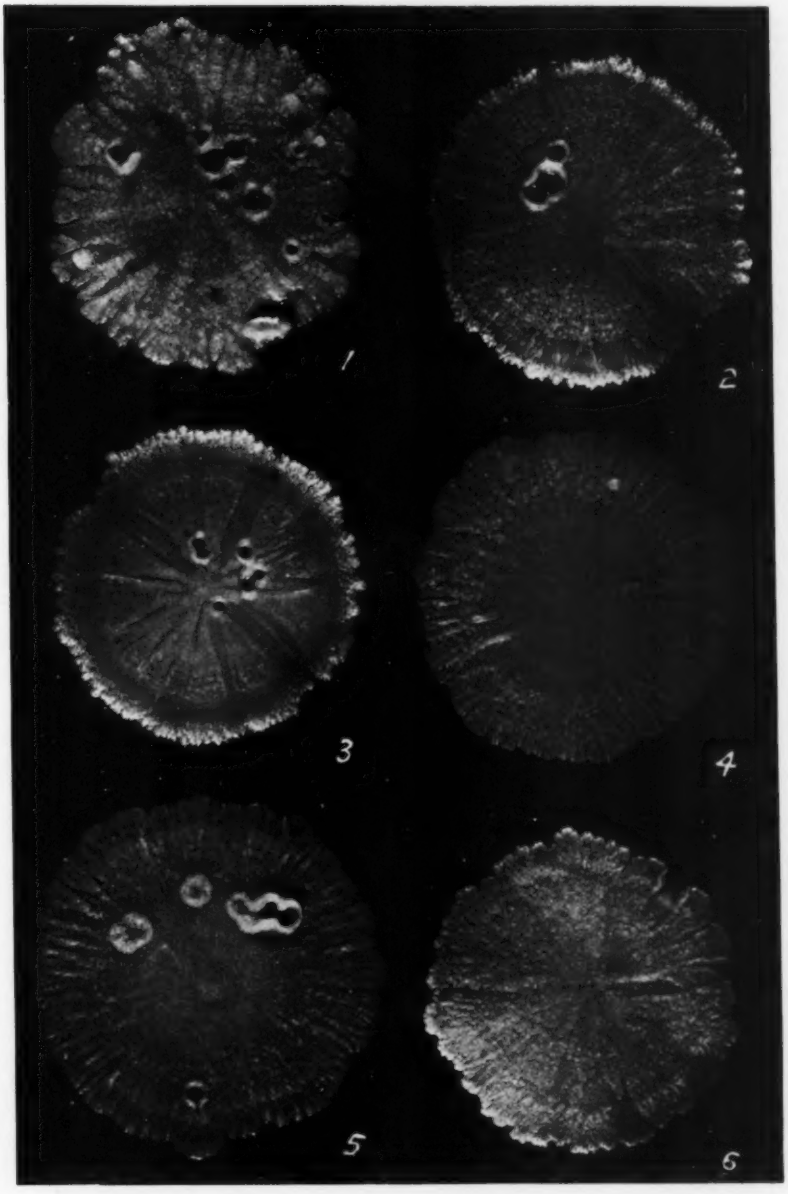
DESCRIPTION OF PHOTOGRAPHS

Photo No.	Strain	Period of growth in days	Diameter of the colony in cm.	Photographed on
1	BY 1	14	3·1	13 -9-1949
2	BY 1S	19	3·1	4-10-1949
3	do.	19	2·6	do.
4	Control 1	13	3·6	13-10-1949
5	Control 2	13	3·2	do.
6	UV 1	19	3·2	4-10-1949
7	do.	20	3·4	5-10-1949
8	UV 2	19	3·4	4-10-1949
9	do.	19	3·5	do.
10	CYG 2	13	3·8	13-10-1949
11	CYG 3	13	3·0	do.
12	CYG 4	13	3·1	do.
13	do.	15	3·0	3-11-1949
14	CYG 6	13	3·3	13-10-1949
15	CYG 7	15	2·6	15-10-1949
16	CYG 8	13	4·0	13-10-1949
17	Control 1	18	2·6	28-11-1949
18	Control 2	18	3·0	do.
19	CYG 1	20	3·9	do.
20	CYG 2	20	2·6	do.
21	CYG 3	20	3·5	do.
22	CYG 4	20	3·3	do.
23	CYG 5	18	2·7	do.
24	CYG 6	21	3·6	1-12-1949
25	CYG 7	17	2·7	28-11-1949
26	CYG 8	16	3·3	9-12-1949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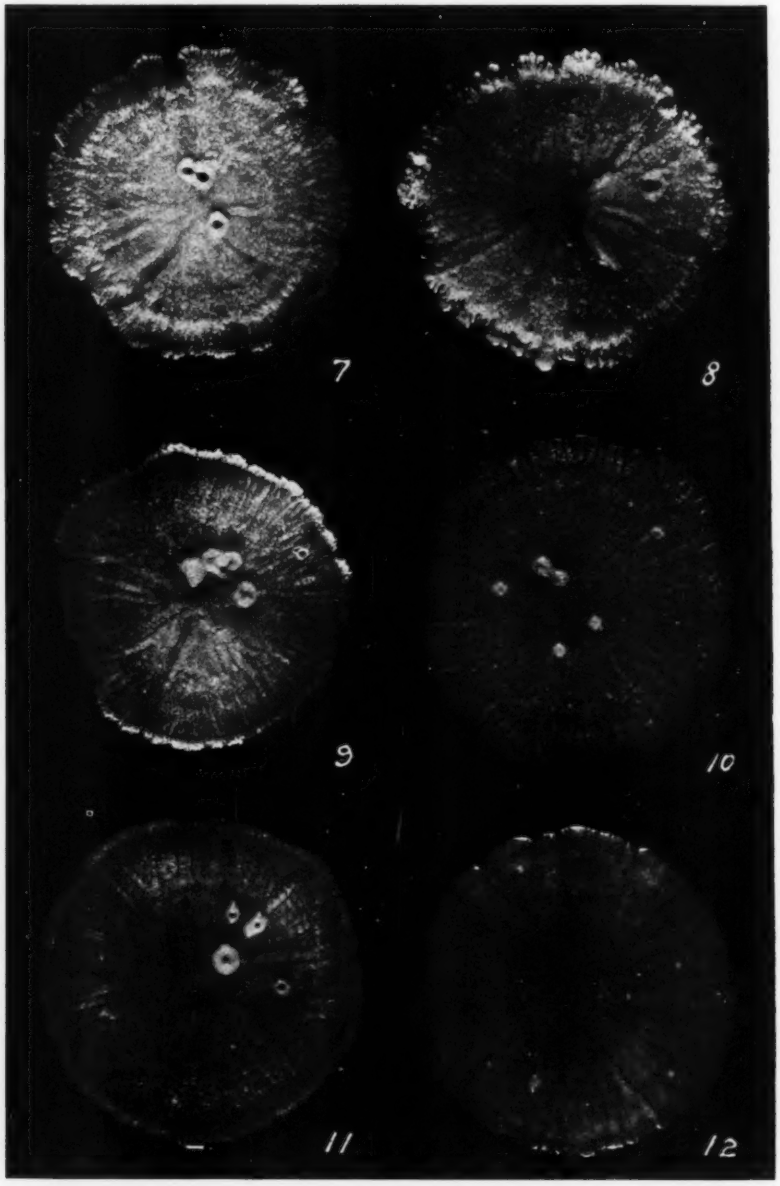
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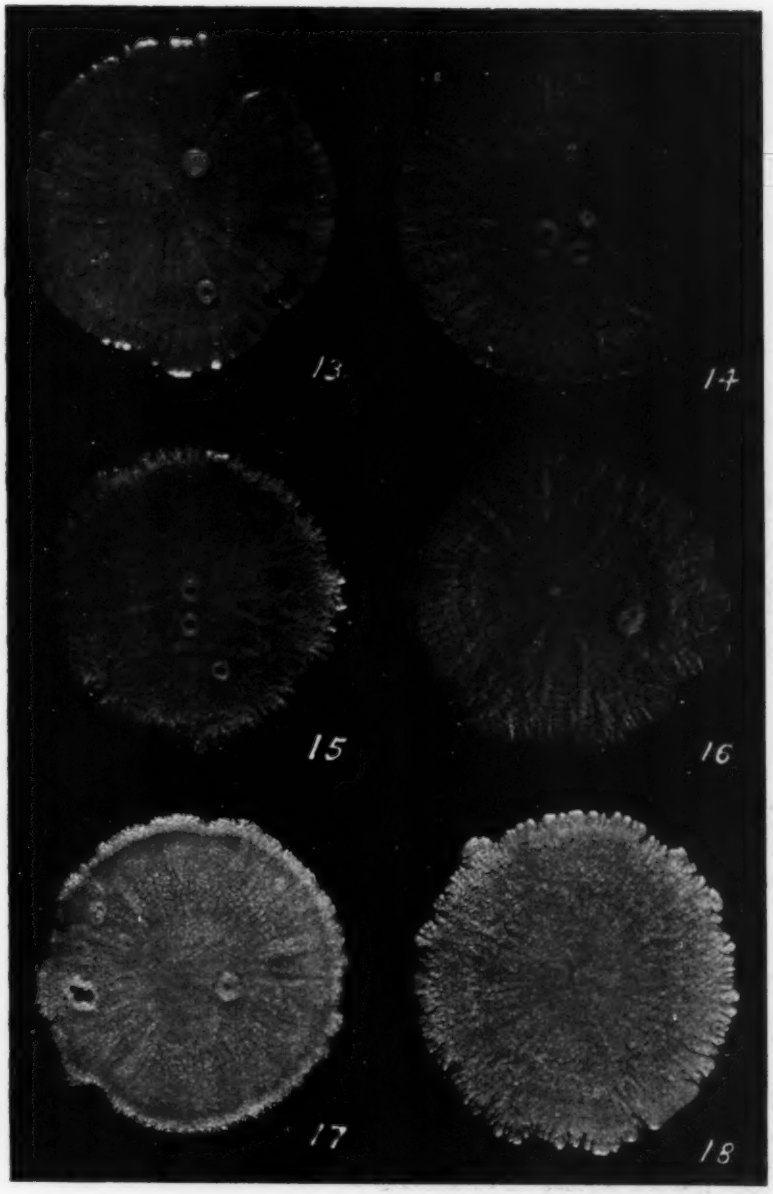
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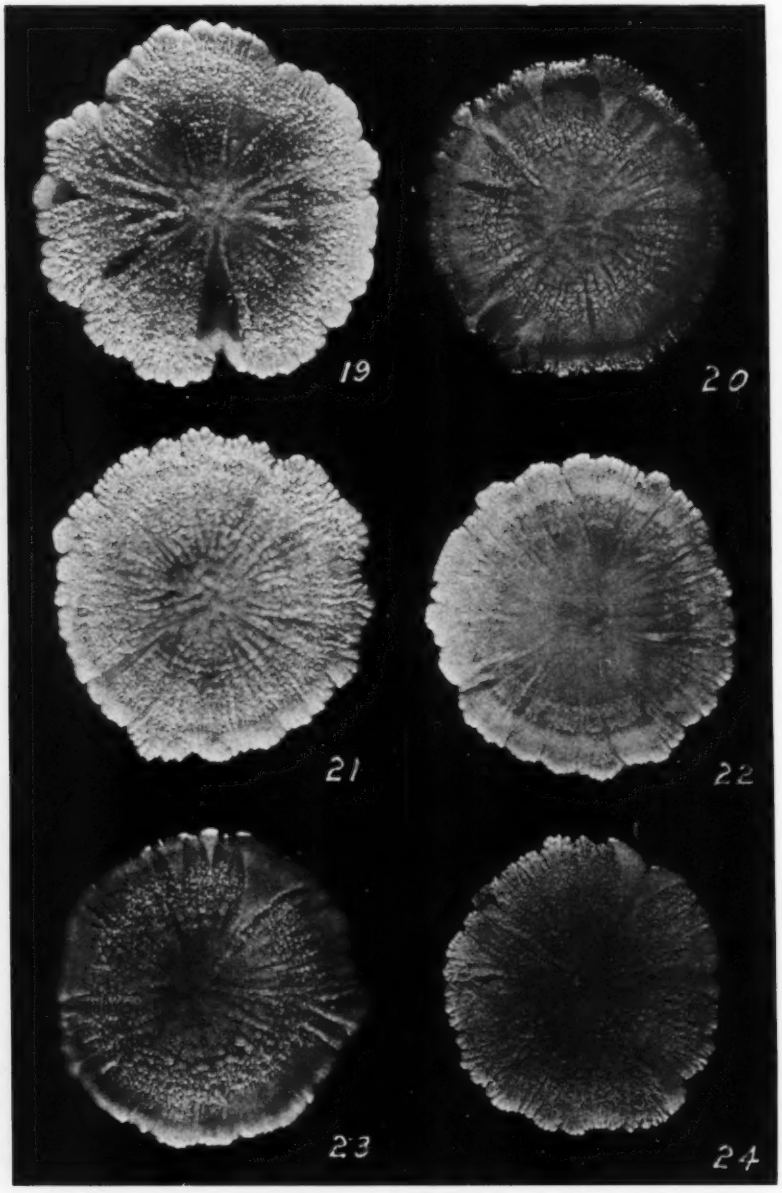
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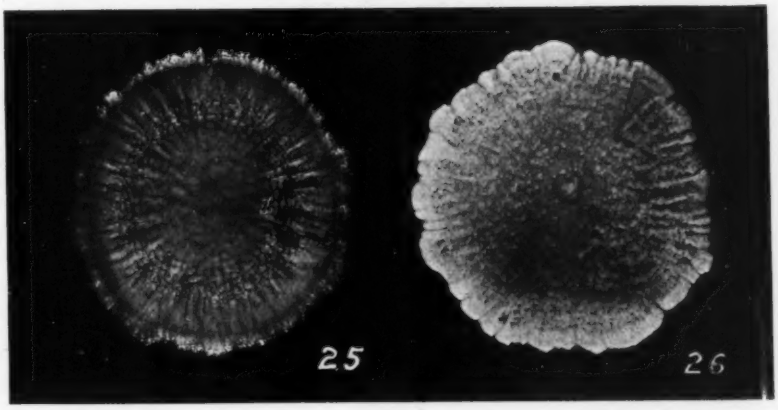
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STIMULATION OF UNSTRIATED MUSCLE BY CHANGE OF TEMPERATURE AND OTHER EFFECTS OF TEMPERATURE

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The reports on the effect of change of temperature on unstriated muscle are conflicting. Cooling may cause unstriated muscle to contract (Botazzi and Grunbaum, 1899; De Zilva, 1901; Starling, 1912; Roskam, 1920 *a*; Verbitzky, 1922; Evans, 1923; Botazzi, 1925; Phillips and Mckenzie, 1934; Hertzman and Roth, 1942; Singh and Singh, 1944 *b*; Perkins, *et al.*, 1950). Cooling may cause relaxation of unstriated muscle (Samkowy, 1874; Roskam, 1920 *b*, *c*; Verbitzky, 1922; Rao and Singh, 1940; Aschoff, 1943). Starling (1912) and Evans (1926) emphasize the fact that rate of cooling is a more effective stimulus than gradual cooling, though the latter also may be effective. The muscle may or may not accommodate to the change of temperature.

These conflicting results are due to the fact that unstriated muscle contains several variables, and the description of the effect of temperature is usually confined to one of these variables. The two main variables are contraction and inhibition. Inhibition is of two kinds; in one the relaxation is active and in the other it is passive. Contractions are of two kinds, tonic and phasic. Tonic contractions are of two kinds; one is susceptible to asphyxia and the other is resistant. The latter tonus is of two kinds; one is susceptible to inhibition and the other is resistant. The last is the basic tone found in all muscles (Singh and Singh, 1949 *a*, *b*; 1951). The twitch contractions are of four kinds produced respectively by alternating current, nervous stimulation, ions and drugs. Inhibition again is of two kinds, tonic and phasic (Singh, 1942). The twitch inhibition is also of four kinds produced respectively by electric current, nervous stimulation, ions and drugs. Singh and Singh (1949 *b*) have shown that agencies which depress excitability, cause contraction of unstriated muscle; cooling belongs to this category of stimulants.

In the present experiments, transverse pieces from frog's stomach muscle (Singh, 1939), from dog's stomach muscle (Singh, 1940) and fowl's gut (Singh, Singh and Muthana, 1947) were used. The muscles were stimu-

lated with alternating current, 10 volts for 10 seconds, potassium or acetylcholine.

RESULTS

Change of temperature produces two kinds of responses. One group of them are functions of a particular temperature; the other group are functions of the rate of changes of temperature. Change of temperature may produce contraction or relaxation which may be temporary or permanent. The latter effects are due to change in the tonus of the muscle and the former effects may be tonic or phasic. To understand these tonic and phasic effects, the permanent effects of temperature or tonus have to be first considered.

Effect on tonus.—The permanent effects are variations in tonus and many of them have been described previously. They are a function of the temperature, and not of the rate of change of temperature.

If frog's stomach muscle is cooled to about 2° C., it relaxes, but dog's stomach muscle at first relaxes and then contracts; for these experiments an isotonic lever should be used. Frog's muscle mainly shows lactic tone, and dog's stomach muscle, alactic tone. Therefore the contractile system which requires energy from chemical stores, relaxes, and the other contractile system, which requires energy for relaxation, contracts due to the action of cold (Singh and Singh, 1949 *b*).

In dog's stomach muscle, the agencies that diminish metabolism, such as asphyxia, cyanide, iodoacetic acid and extreme cold, first cause the muscle to relax and then cause contraction; the relaxation is due to the action on the first contractile mechanism, and contraction due to action on the second contractile mechanism. In frog's stomach muscle the action is predominantly that of relaxation, as the second contractile system is feeble, though not entirely absent, as the above agencies may produce a feeble contraction (Singh and Singh, 1948). In dog's stomach muscle, the preliminary relaxation is sometimes absent, and relaxation on warming is active (Singh and Singh, 1949 *b*). When dog's stomach muscle is put under tension at low temperatures, on warming up to 25° C., it relaxes. On recooling its contraction is very feeble. An unloaded dog's stomach contracts when cooled (Singh and Singh, 1949 *b*).

In the frog's stomach muscle, the tonus curve shows a maximum at 20° and 45° C., the tone beginning to increase after 35° C. (Rao and Singh, 1940). Therefore if a muscle is cooled below 20° C. or warmed above 35° C. either gradually or suddenly it may show a tonic contraction. The tonus at 20° C. is susceptible to asphyxia; therefore, it is lactic tone. This is

supported by oxygen consumption experiments (Rao and Singh, 1940). The contraction above 35° C. is also lactic tone, as it is susceptible to asphyxia and is diminished by iodoacetic acid. If some of the sodium of the saline is replaced with potassium or the chloride with other stimulating anions, such as bromide, nitrate, iodide, thiocyanate, then the tone increases continuously with temperature, as these substances antagonise adaptation and the decline of tone after 20° C. being due to adaptation (Rao and Singh, 1940). The tonus produced by these anions is lactic tone, as it is diminished by asphyxia. Thus frog's muscle predominantly shows lactic tone.

Mytilus muscle also shows maximum at about 15 to 20 and 45° C. Dog's stomach muscle behaves differently. If it is put under stretch, so that it exerts tension at about 10° C., and is then gradually warmed, it relaxes upto 25–30° C.; thereafter it begins to contract. The tone at high temperatures is not susceptible to asphyxia, and may pass into that stage when it is not susceptible to inhibition. So these are the reactions of alactic tone. Lactic tone can be induced in dog's stomach muscle by replacing some of the sodium of the saline with potassium or barium, or by replacing the chloride with other anions such as iodide, thiocyanate. Tonus then shows a maximum at about 30° C.; it may continue to increase up to 37–40° C., when it passes into the alactic stage (Fig. 1). In the fowl's gut and human appendix, the tone continues to increase with temperature up to 37–40° C., and is susceptible to asphyxia (Singh, Singh and Muthana, 1947; Khan and Singh, 1947).

Some of the above effects on tonus of unstriated muscle have also been observed on isolated proteins. Varga (1946) has observed that actomyosin threads shorten maximally at 16–18° C. Other similarities between isolated proteins and muscle have been described previously (Singh, 1943; Singh and Singh, 1951). Astbury and Dickinson (1940) have shown that isolated strips of myosin from unstriated muscle show thermal contraction below 40° C.

Optimum temperature for twitch responses.—This has been described by Winton (1926) and others (Singh, 1940; 1942, 1944 *a*; Rao and Singh, 1940; Narayana and Singh, 1944, Singh, Singh and Muthana, 1947; Khan and Singh, 1947). In frog's muscle it has been found, that the optimum temperature is a function of the surrounding temperature, that is, the tissue exhibits adaptation or acclimatisation; this is found in mammalian and avian muscle also to some extent, as shown in the above papers.

Frog's stomach muscle was stimulated by alternating current (8 volts for 10 seconds every 10–15 minutes), potassium (20 p.c. of the sodium of

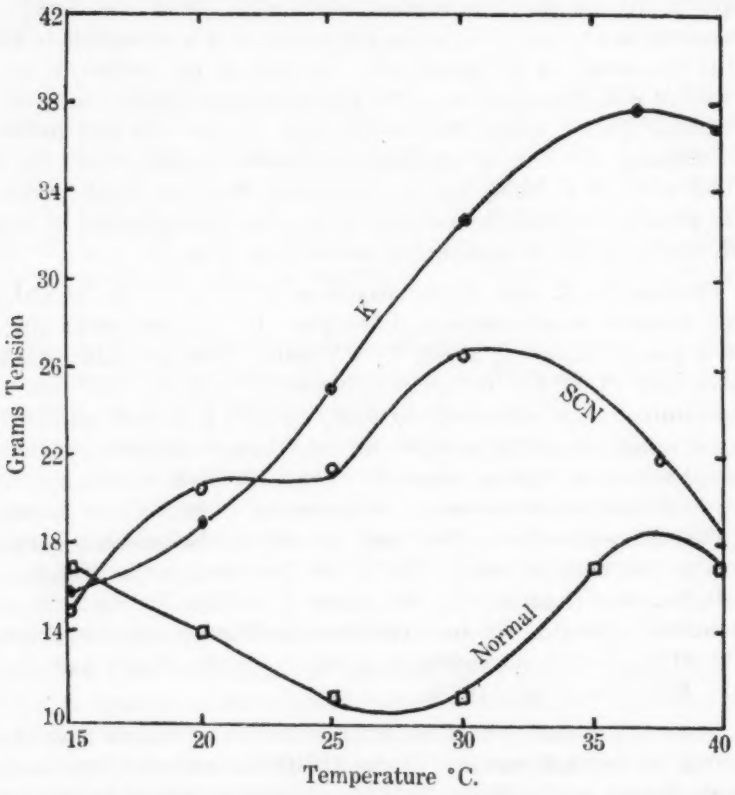

FIG. 1. Dog's stomach muscle. Effect of temperature on normal tone and tone produced by potassium (40 p.c. of the sodium of the saline replaced with potassium) and thiocyanate (chloride of the saline replaced with thiocyanate).

the saline being replaced with potassium) and acetylcholine (1 in 5,000). Two methods were employed; in the first method, the temperature was gradually raised to 30–35° C. and then gradually lowered back agian. In the second method, the temperature was raised gradually and then suddenly lowered to the original value, and after an hour or so the observations were repeated.

When the temperature is increased, the response to alternating current at first increases and then declines (12 experiments); when the temperature is lowered, the opposite happens (Fig. 2). This shows that the muscle tries to counteract the effect of temperature. Increase of temperature would hasten the chemical processes, and so increase the response, but an inhi-

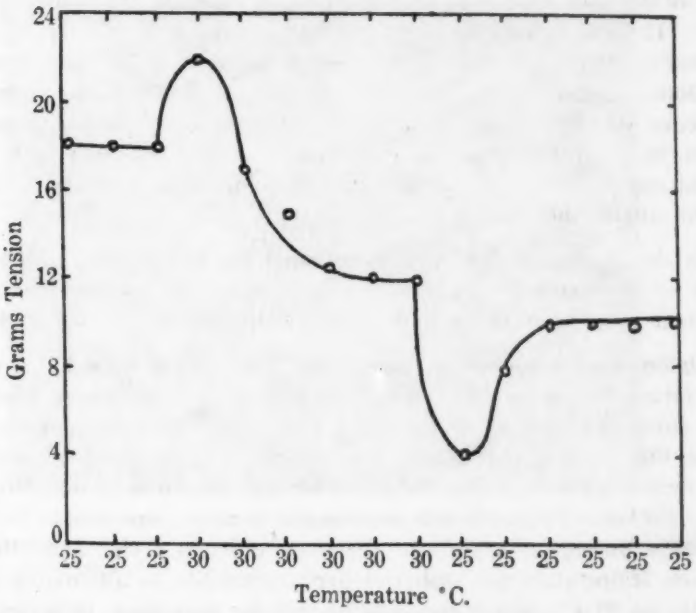

FIG. 2. Frog's stomach muscle. Effect of temperature on the response to alternating current, 6 volts in 10 seconds every 15 minutes.

bitory influence sets in to oppose this action. With decrease of temperature the chemical processes would diminish, but some accelerating influence sets in so that the muscle recovers.

The above temporary increase of excitability on increase of temperature can be produced by increase in the concentration of calcium in the saline by about 3 to 6-fold, and the opposite effects can be produced by decrease in the concentration of calcium. Increase of temperature appears to liberate calcium (Singh, 1944 *a*; Rao and Singh, 1940). It is probable that in protoplasm, the dissociation of calcium is a function of temperature. The above increase in excitability can be produced to a less extent by increase of potassium and sodium ions in the saline, potassium about two-fold and sodium by about 40 p.c. by addition of sodium chloride to the saline, but the opposite effect has not been observed with these ions, so that the change in excitability due to change of temperature are probably a calcium effect.

If the temperature of frog's stomach muscle is gradually increased, then again gradually lowered, the optimum temperature may increase (Fig. 2). This especially happens if the tmeperature is raised high, such as 35° C. The

cause of this shift appears to be adaptation, as shown by the following experiment. The temperature is at first gradually increased as before, but instead of decreasing the temperature back again to its original value gradually, it is done suddenly and the muscle is allowed to remain at this temperature for about an hour or so. It is then found that the optimum temperature retains its original value. Six experiments were performed with acetylcholine and four with potassium; the alterations in excitability were noted as with alternating current.

In dog's stomach muscle, the optimum temperature for twitches produced by alternating current, potassium, acetylcholine, nervous stimulation and lactic tone, is about 25–30° C.; for alactic tone it is about 37–40° C.

Optimum temperature for asphyxial hyperexcitability.—The optimum temperature for the asphyxial hyperexcitability to alternating current in frog's stomach muscle is 30° C. (Singh and Singh, 1948). In dog's stomach muscle, the optimum temperature for asphyxial hyperexcitability to alternating current is 20–30° C.; that for potassium and tone it is 20° C. (Singh and Singh, 1949 *a*). The metabolic mechanisms for the responses to these two stimulants during asphyxia are therefore different. The fact that the optimum temperature for asphyxial hyperexcitability to alternating current can also be 20° C., that is the same as that for potassium, indicates that a common metabolic mechanism may subserve the response to both potassium and alternating current during asphyxia. The optimum temperature for asphyxial hyperexcitability should be kept in mind, as it may not be obtained at 37° C., the temperature at which mammalian muscles are usually studied.

Rhythmic stimulation by increase of temperature.—Increase of temperature produces a very interesting effect. If the temperature of the saline is gradually raised at the rate of $\frac{1}{2}$ to 1° C. per minute from 15 to 20° C., or from 20 to 30° C., or from 30 to 37° C., dog's stomach muscle executes powerful rhythmic contractions, about one per minute, and lasting for about 10 minutes, before it settles down to a steady state during which the rhythmic contractions are only about 5 to 10 p.c. of the previous ones (Fig. 3). Same phenomenon is produced by alternating current with about 10 volts per second every minute. The above contractions are produced by change of temperature as shown by the fact that they are absent if the temperature is raised very gradually. about 1° C. in about 10 minutes, the minimum rate at which the temperature produces the rhythmic state being about 2° C. rise in 10 minutes (6 experiments). This is also shown by the fact, that if the rhythmic contractions are not produced by rise of temperature at the rate of 1° C. per minute, they may be produced if the rate of rise of temperature is

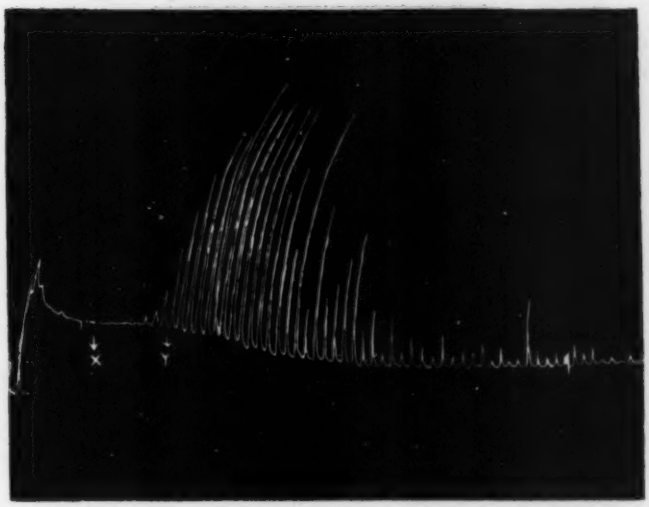

FIG. 3. Dog's stomach muscle. Rhythmic stimulation of dog's stomach muscle by increase of temperature from 15° C. to 25° C. at the rate of 1 °C. rise per minute. Warming begins at X and finishes at Y.

more rapid, about 2° C. per minute. These contractions are increased by 0·1 p.c. glucose. These rhythmic contractions are not the same as those produced in frog's muscle, as no corresponding decrease in rhythmic contractions have been noticed in dog's stomach muscle on decrease of temperature.

The above rhythmic contractions resemble those produced during asphyxial hyperexcitability, or when glucose is added to a muscle which has been asphyxiated or treated with cyanide. Glucose produces a temporary increase in excitability (Singh and Singh, 1949 *c*, 1949 *d*). These experiments suggest that during asphyxia, or increase of temperature a new metabolic mechanism is brought into action.

Temporary contractions.—Besides the contractions which may result by change of temperature described above, other tonic and twitch contractions are also produced.

Change of temperature produces a twitch contraction in dog's and frog's stomach muscle (Fig. 4). Both sudden heating and cooling produces the contraction. The muscles show wide variations in excitability to change of temperature. Some muscles do not show any response, whilst others are quite sensitive. With dog's stomach muscle, the

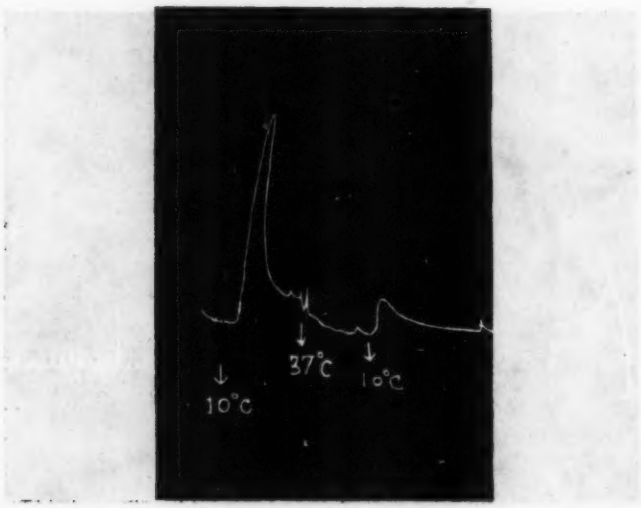

FIG. 4. Dog's stomach muscle. Twitch contraction produced by sudden cooling. Initial temperature 25° C.

sensitivity is greatest in freshly dissected muscles, as happens with excitability to other forms of stimulation. This is probably due to the fact, that with keeping glycogen breaks down into lactic acid and thus the energy reserves are diminished (Bharadwaj and Singh, 1951).

The muscle may be stimulated by different variations of temperature. Thus it may be cooled from 20 to 5° C., or 35 to 20° C. and also warmed similarly. Generally the muscles are more sensitive to cooling, though at other times they are more sensitive to warming. The sensitivity to cooling and warming may vary in opposite directions. If a certain change of temperature does not cause contraction, a greater change may be effective. Relaxation of contraction produced by warming is much rapid than that produced by cooling. If the muscle is stimulated by warming, it usually gives the maximum response if the temperature is raised from 15° C. (6 experiments). If the muscle is cooled below 15° C., then the response declines (Fig. 5). This is due to the fact, that excessive cooling lowers the excitability of the muscle, so that though change in temperature causes stimulation, to do so, the muscle must be excitable. The muscle may adapt to low temperature so that with frequent cooling it may become more excitable; then the muscle may be cooled below 15° C. (experimental range, 5° C.), and then warming causes a maximum response. Similarly if stimulated by cooling, the response may decline if the muscle is cooled below a

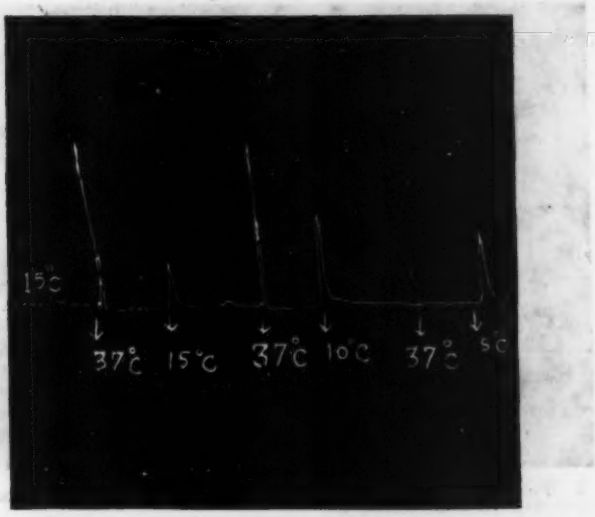

FIG. 5. Dog's stomach muscle. Contractions produced by sudden warming and cooling. Initial temperature 15° C.

certain temperature (in dog's muscle, 10° C.) as found by Perkins, *et al.* (1951). This then will be due to depression of excitability by excessive cooling. In hyperexcitable muscles, the muscle may be cooled below 10° C., without decline of the response.

Though a tonic contraction may not be produced on cooling, after a twitch due to cooling, there may be left residual tone on subsidence of the twitch contraction.

Tonic contraction is also produced on change of temperature in those muscles which show lactic tone, such as frog's stomach muscle (Fig. 6). In dog's stomach muscle which shows alactic tone, lactic tone can be induced by potassium, or anions such as iodide, thiocyanate, and the muscle then shows a tonic contraction on change of temperature.

Cause of thermal contraction.—One way in which change of temperature acts is to lower the threshold of excitability to ions outside the muscle fibres; the change of temperature sensitises the muscle to ions, as is done by stretching, drugs, touching and electrical stimulation (Singh, 1938 *a*, *b*, 1944 *b*, 1945; Singh and Singh, 1947). This is shown by the following experiments: (1) Simultaneous excitation of dog's or frog's muscle by heating or cooling and potassium potentiates the response to the latter (Fig. 7). (2) If the muscle is inexcitable to potassium, then sudden heating

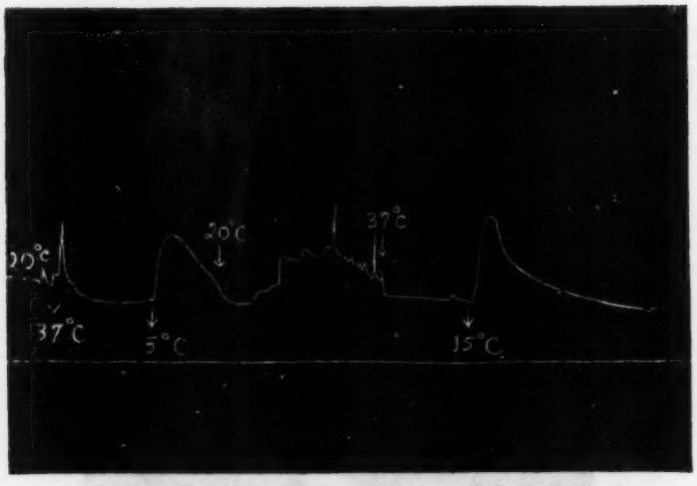

FIG. 6. Frog's stomach muscle. Tonic contraction produced by cooling.

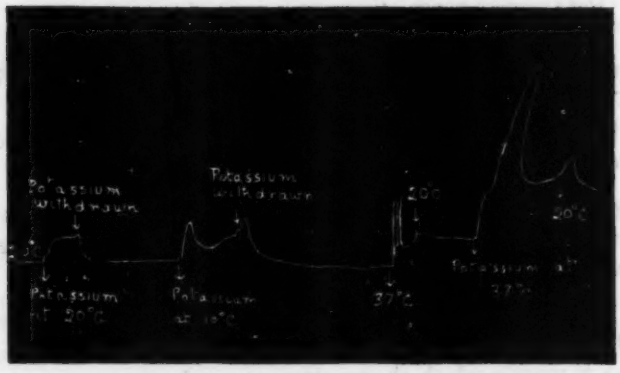

FIG. 7. Dog's stomach muscle. Normal response to potassium at 20° C. (20 p.c. of the sodium of the saline replaced with patassium). Addition of potassium saline the temperature of which was 10° C. Initial temperature 20° C. Potassium removed. Temperature of saline increased to 37° C. Saline added at 20° C. Potassium saline at 37° C added. Note that both cooling and warming potentiate the response to potassium.

or cooling enables potassium to act, and produces a tonic contraction in dog's muscle (Fig. 8). (3) The response to change of temperature in dog's stomach muscle is potentiated by excess of calcium, as is the response to potassium (Singh, 1940).

For the production of thermal contractions, the excitability of the muscle must be normal. Thus excess of potassium in frog's muscle

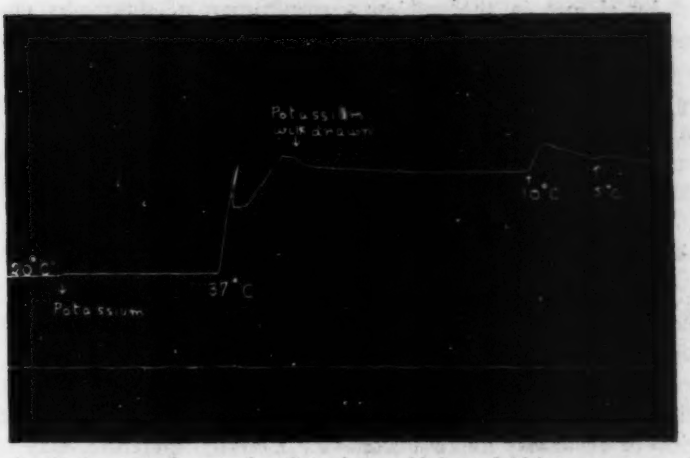

FIG. 8. Dog's stomach muscle. Potassium added at initial temperature of 20° C Temperature then suddenly increased to 37° C. Potassium withdrawn and temperature decreased to 10 and 5° C. (20 p.c. of the sodium of the saline replaced with potassium to cause stimulation).

depresses excitability and also the thermal response; in dog's muscle, excess of hydrogen ions beyond pH 6·5 depress the response. The twitch contractions are susceptible to asphyxia, and the tonic contractions in excess of potassium described above may or may not be susceptible, as happens with the potassium contraction.

In the guinea pig's uterus, most agencies that cause excitation in other muscles cause inhibition (Singh, 1942). It is interesting to note, therefore, that in this muscle change of temperature may produce an inhibitory twitch. The pH of the saline was 8 (borate buffer) and temperature was increased from 30° C. to 38° C. suddenly or lowered from 38° C. to 25° C. As the tone declines with decrease of temperature, the inhibitory twitch on sudden cooling is followed by slow decline of tone, so that the sequence of events on sudden cooling are as follows: first, there is relaxation and then contraction which together constitute the inhibitoty twitch; this is followed by slow decline of tone. Greater cooling tends to cause contraction, though in some muscles an inhibitory twitch is produced, even if the temperature is suddenly lowered from 38° C. to 5° C.

DISCUSSION

Change of temperature probably affects the muscle membrane, making it more permeable or affects its calcium concentration. The increased sensitivity to ions induced by change of temperature, is similar to that

induced by change in the calcium concentration of saline, by change of tension or by drugs. It is known that mere presence of ions outside the muscle membrane is not sufficient to cause excitation. Thus *Mytilus* muscle may be immersed in a barium-rich saline, without any contraction resulting. Barium will produce its typical response if the muscle is suddenly stretched or released, or if calcium is excluded from the saline, or if electric current is passed for a second, or if drugs such as adrenaline or acetylcholine are added. Similarly, potassium can be present in the saline without stimulating dog's muscle, but stimulation occurs on sudden change of temperature, or by the above procedures.

The ions normally present in the saline in predominant numbers are the sodium or chloride ions, and these are likely to cause excitation. Both inside and outside the muscle there are inhibitory or excitatory ions. Excitation can be caused if the action of inhibitory ions is suppressed or if the action of excitatory ions is increased. Cooling, therefore, may suppress the action of inhibitory ions, thus causing excitation (Singh and Singh, 1949 *b*), and heating may increase the action of excitatory ions; the latter is actually shown to occur in Fig. 8.

During change of temperature, ions from the saline probably move into an outer zone to cause excitation (Singh, 1944 *b*).

SUMMARY

1. Temperature produces two kinds of effect on unstriated muscle. One of these is a function of a particular temperature and the other is a function of the rate of change of temperature. The former produces changes in tonus, and the latter produces both tonic and phasic effects.

2. Excessive cooling increases tonus of muscles showing alactic tone, and causes relaxation of muscles showing lactic tone.

3. Temperature affects lactic and alactic tones differently. Lactic tone increases with temperature up to 30–37° C. Alactic tone at first decreases with temperature up to 25–30° C. and then increases.

4. The optimum temperature for tonus and twitch responses is different.

5. Frog's stomach muscle shows adaptation to temperature.

6. In dog's stomach muscle, the optimum temperature for asphyxial hyperexictability is 20° C. for potassium, and 20 to 30° C. for alternating current.

7. Increase of temperature at the rate of 2 to 10° C. per minute causes a temporary rhythmic stimulation of dog's stomach muscle. This rhythmic

stimulation resembles similar stimulation by asphyxia or by glucose in an asphyxiated muscle.

8. Change of temperature at certain rate produces both a tonic and phasic contraction of unstriated muscle.

9. Change of temperature sensitises the muscle to ions outside the muscle fibres.

10. Cooling and warming may cause contraction by direct action on the contractile mechanism.

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